1975

An investigation into the mode of action of alachlor.

James Russell Bates
University of Massachusetts Amherst

AN INVESTIGATION INTO THE MODE OF ACTION OF ALACHLOR

A Thesis Presented

By

James Russell Bates

Submitted to the Graduate School of the
University of Massachusetts in partial fulfillment
of the requirements for the degree of

MASTER OF SCIENCE

December 1975

Department of Plant and Soil Sciences

AN INVESTIGATION INTO THE MODE OF ACTION OF ALACHLOR

A Master's Thesis Presented

By

James Russell Bates

Approved as to style and content by:

Herbert V. Marsh, Jr., Chairperson of Committee

Bernard Rubinstein, Member

Donald L. Schneider, Member

Frank W. Southwick, Dept. Head
Plant and Soil Sciences

ABSTRACT

An Investigation Into The Mode of
Action of Alachlor

(December 1975)

James R. Bates, B.S., University of Massachusetts
M.A., University of Massachusetts

Directed by: Professor Herbert V. Marsh Jr.

Evidence is presented that 2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide (alachlor) inhibits auxin-promoted elongation of Avena coleoptile sections and auxin-promoted hydrogen ion secretion from these sections. Using 0.1 mM alachlor, the onset of inhibition in both cases occurred between three and four hours after treatment. Further investigation revealed that 0.1 mM alachlor strongly inhibited auxin transport in Avena coleoptiles, which may explain alachlor's effect on growth and the growth related hydrogen ion secretion.

Morphological observations on the effects of alachlor on oat roots indicated a strong inhibition of growth of primary and lateral roots. Anatomical examination of the region of cellular expansion showed a pronounced inhibition of cortical cell enlargement. Examination of mitotic indices showed that alachlor may also interfere with cell division. A time course of increase in fresh and dry weights of oat seedlings showed an inhibition by alachlor of increase in fresh weight of roots preceding by a day a similar inhibition

in the shoots. Increases in dry weight were inhibited by alachlor in the roots only, no inhibition of increase in dry weight was noted in the shoots.

Alachlor at a concentration of 0.1 mM inhibited uptake of leucine, alpha-amino isobutryic acid, 3-O-methyl glucose and potassium but not calcium. Inhibition of leucine uptake was noted within 30 minutes. Examination of leucine incorporation into protein indicated that alachlor does not inhibit this process. In addition, no effect of alachlor on respiration or on membrane leakage could be detected.

Alachlor has been shown to severely inhibit the root and shoot growth of zero to 3-day old corn seedlings growing in sand when applied by subirragation. The roots of 5-day old seedlings were inhibited by the same treatment, but shoots of the older seedlings showed no response. The coleoptile of corn is apparently insensitive to alachlor in that the herbicide neither inhibited elongation of coleoptile segments nor ^{14}C-leucine uptake by this tissue. Leucine uptake by excised roots, however, was inhibited 40 to 67%.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

SECTION III

SECTION IV

SECTION I

THE EFFECT OF ALACHLOR ON AUXIN PROMOTED GROWTH IN *AVENA* COLEOPTILES

Abstract

Evidence is presented that 2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide (alachlor) inhibits auxin-promoted elongation of Avena coleoptile sections and auxin-promoted hydrogen ion secretion from these sections. Using 0.1 mM alachlor, the onset of inhibition in both cases occurred between three and four hours after treatment. Further investigation revealed that 0.1 mM alachlor strongly inhibited auxin transport in Avena coleoptiles, which may explain alachlor's effect on growth and the growth related hydrogen ion secretion.

Introduction

Alachlor inhibition of the growth of Avena seedlings has been previously reported (3). More recently reported is the inhibition of auxin-induced and acid-induced elongation of Avena coleoptile sections by alachlor (1). Hydrogen ion secretion in response to auxin has been shown in sunflower hycotyls (5) and in oat coleoptiles (4, 6). These observations support the concept that hydrogen ions act as a second messenger in auxin-induced cell elongation. It will be shown here that alachlor interferes with the auxin-promoted hydrogen ion secretion, that this interference is probably brought about by the inhibition of auxin transport, and that it may explain in part the

alachlor inhibition of growth.

Materials and Methods

Plant material: In all experiments the plant material consisted of coleoptile tissue of Avena sativa varr. orbit. The Avena seedlings were grown on vermiculite in plastic boxes. The boxes were kept at room temperature in the dark for four days at which time the seedlings were harvested. All experiments were carried out under incandescent room lighting.

Growth tests: Sections (7.6 mm) of isolated coleoptiles were cut approximately 3mm from the tip. Sections were then placed in plastic dishes containing 10 mM potassium phosphate buffer (pH 6.5) with and without 0.1 mM alachlor. Growth measurements were taken periodically using a dissection microscope and a lens micrometer.

H^+ secretion assay: Isolated coleoptile sections (10 mm) were cut approximately 3 mm from the tip. The cuticle and adhering outer epidermal cell wall was stripped off using fine forceps according to the procedure of Cleland (4). No visible damage to cortical cells was noted under microscopic examination. The sections were floated in 1 mM potassium phosphate (pH 5.9) for 30 minutes and then 15 each were placed into 25 ml beakers which contained 3 ml of 1 mM potassium phosphate (pH 5.9) with and without 0.1 mM alachlor. Small magnetic spin bars were placed in

the beakers for agitation. Hydrogen ion secretion was monitored using a combination pH electrode and an expanded scale Beckman pH meter.

Auxin transport assay: Sections (3 mm) of isolated coleoptiles were cut approximately 5 mm from the tip and floated for 3 hours in distilled water containing 10 mM potassium phosphate (pH 6.5) with and without 0.1 mM alachlor. Sections, in groups of four each, were placed upright on 1% agar blocks. A second agar block containing 20 μM ^{14}C-1-IAA (51 Ci/mM) was then placed on top of the sections for one hour. Receiver blocks were collected after a one hour transport period, placed in scintillation fluid (Beckman Ready-Solv solution VI) and counted on a Beckman scintillation counter.

Results and Discussion

The results shown in Figure I concur with those of Bardzik (1). Using 0.1 mM alachlor there is an inhibition of the auxin-promoted elongation which is first apparent at three to four hours after treatment. Inhibition is almost complete by four hours. When the concentration of alachlor is doubled the lag time prior to inhibition is cut in half, indicating that a threshold concentration of alachlor within the tissue is necessary for onset of inhibition.

Results shown in Figure 2 demonstrate the auxin-promoted hydrogen ion secretion reported for Avena

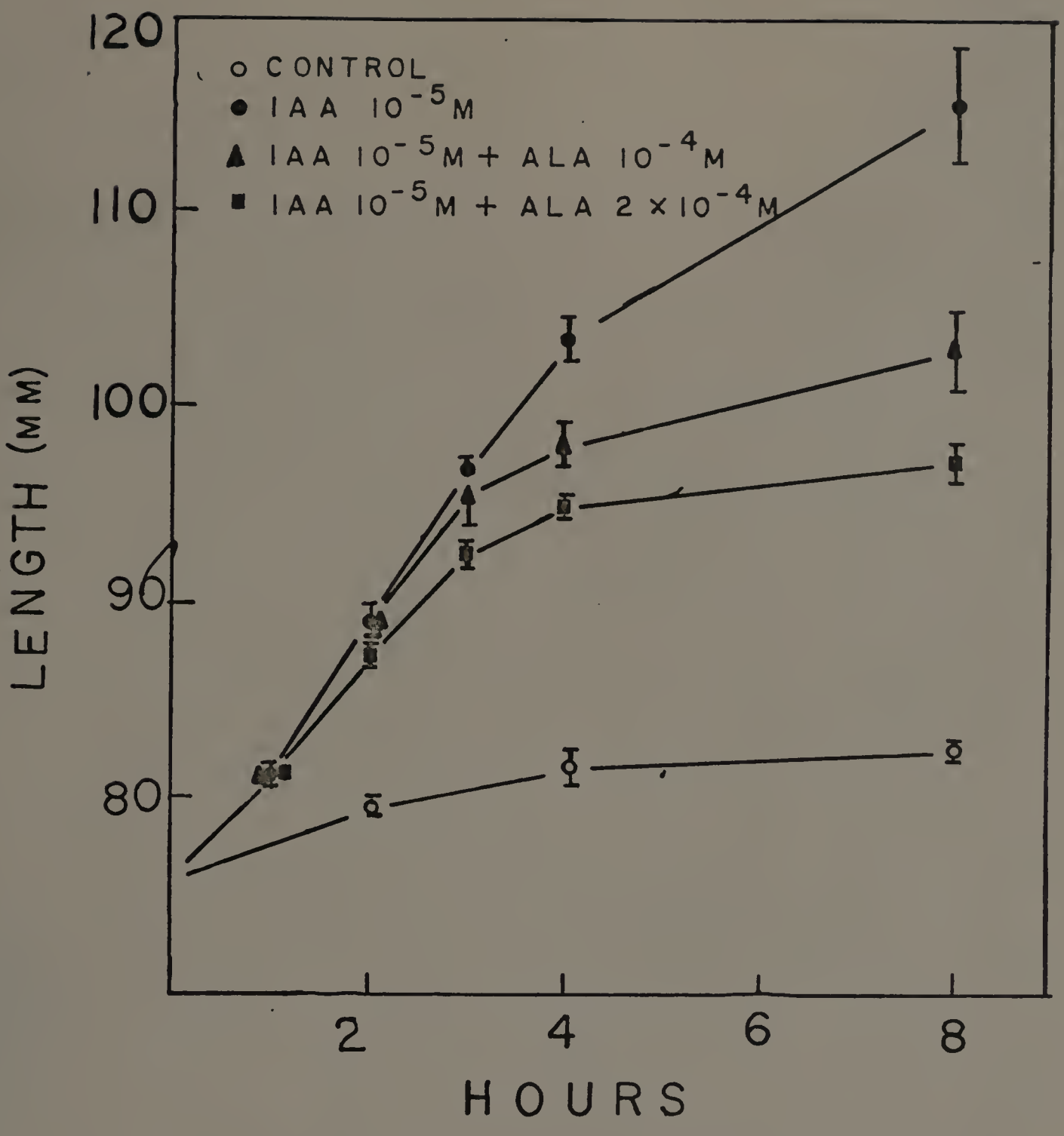

Figure 1. Time course of auxin-promoted elongation in oat coleoptiles with and without alachlor.

coleoptiles. The addition of 0.1 mM alachlor is shown to block the secretion of hydrogen ions. A lag before hydrogen ion secretion due to the auxin treatment becomes apparent has been noted by others (4, 6). Additional tests showed that peeled coleoptiles do grow in response to auxin (data not shown). If the concept (4, 6) that these secreted hydrogen

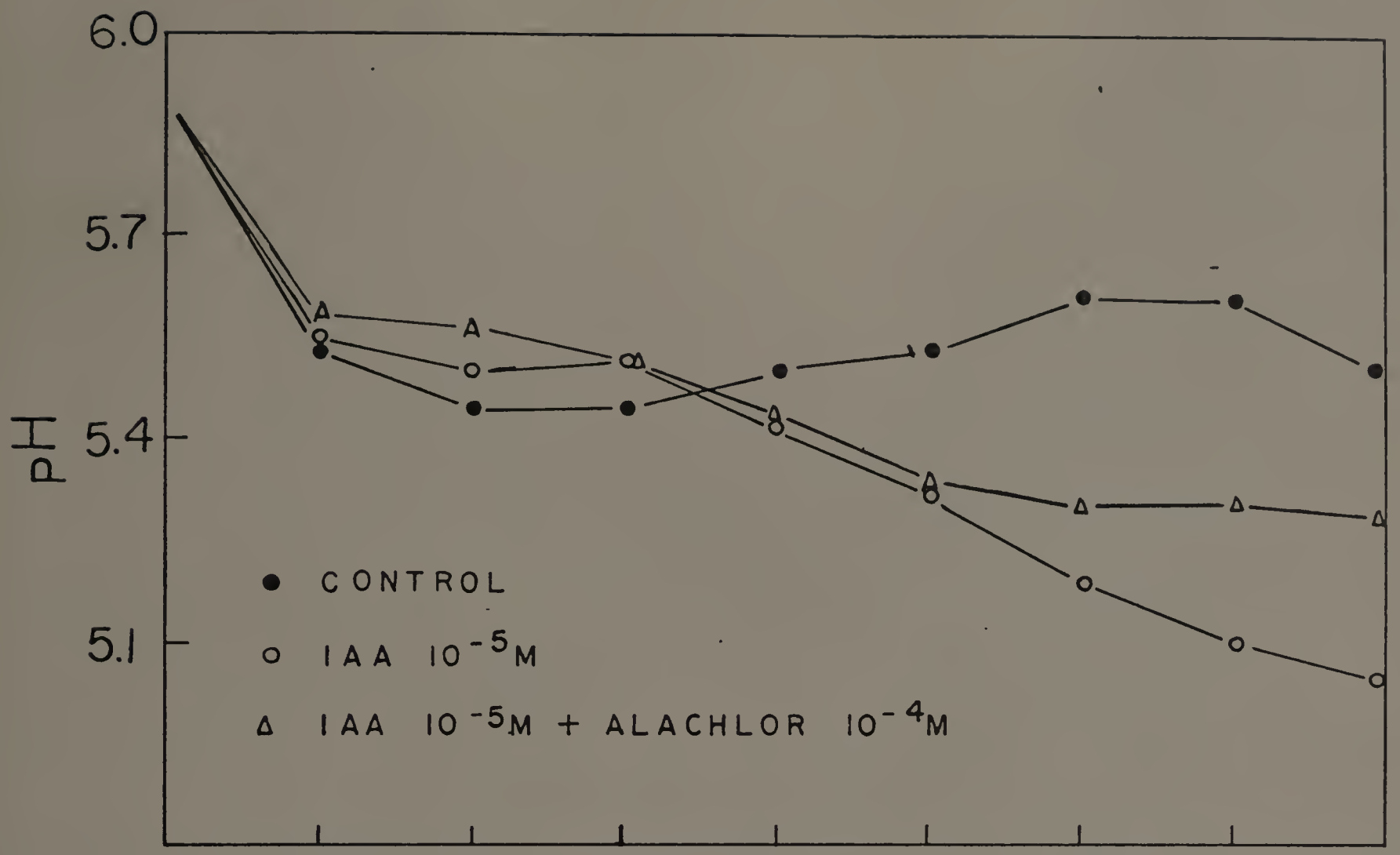

Figure 2. Time course of auxin-promoted H^+ secretion with and without 0.1 mM alachlor.

ions act as a messenger for auxin by acting as a cell wall loosening agent is valid, than this could explain the alachlor inhibition of auxin-promoted growth shown here. The time of onset of inhibition for hydrogen ion secretion is similar to the time of onset of alachlor-induced inhibition of auxin-promoted growth. This indicates that the inhibition of growth may be closely tied to and result from the inhibition of hydrogen ion secretion.

Additional experiments were conducted to determine if

TABLE I

IAA Transport by Avena Coleoptile Sections

TREATMENT	I	II	III
Control (3 hr)	152.7 ±14	2226 ±598	418 ±86
Ala 10^{-4}M (3 hr)	76.6 ±14	2123 ±214	274 ±49

I - cpm/recieving block
II - cpm/apical 1.5 mm/4 sect.
III - cpm/basal 1.5 mm/4 sect.

auxin movement was being affected. Table I shows the effects of 0.1 mM alachlor on auxin transport in Avena coleoptile sections. The inhibition of auxin transport by alachlor is probably a membrane related event. Bardzik (1) reported alachlor inhibition of chloride ion uptake in Avena coleoptiles. Recent reports of alachlor inhibition of uptake of a broad range of solutes in Avena root sections (2) indicate that the inhibition of auxin transport by alachlor may be due to inhibited membrane transport systems. This inhibition of solute uptake, by effecting osmotic pressure of the cells, could result in a limited ability for water uptake thereby restricting growth and explaining the apparent inhibition of acid-induced growth.

Literature Cited

1. Bardzik, J. M. 1974. An investigation of the metabolic and physiological effects of alachlor on Avena seedlings and coleoptile sections. Ph.D. Thesis. Univ. of Mass., Amherst.
2. Bates, J. R. 1975. An investigation into the mode of action of alachlor. Master's Thesis. Univ. of Mass., Amherst.
3. Chang, T. 1973. Effects of alachlor on Avena seedlings. Ph.D. Thesis. Univ. of Mass., Amherst.
4. Cleland, R. 1973. Auxin-induced H^+ ion secretion from Avena coleoptiles. Proc. Nat. Acad. Sci. 70:3092-3093
5. Ilan, I. 1973. On auxin-induced pH drop and on the improbability of its involvement in the primary mechanism of auxin-induced growth promotion. Physiol. Plant. 28:146-148
6. Rayle, D. L. 1973. Auxin-induced H^+ ion secretion in Avena coleoptiles and its implications. Planta 114:63-73

SECTION II

MORPHOLOGICAL AND ANATOMICAL EFFECTS OF ALACHLOR ON OAT ROOTS

Abstract

Morphological observations on the effects of the herbicide, 2-chlor-2',6'-diethyl-N-(methoxymethyl)acetanilide (alachlor), on oat roots (Avena sativa cv. Orbit) indicated a strong inhibition of growth of primary and lateral roots. Anatomical examination of the region of cellular expansion showed a pronounced inhibition of cortical cell enlargement. Examination of mitotic indices showed that alachlor may also interfere with cell division.

Introduction

Previous investigations of the herbicidal activity of alachlor by Eshel (7) and Chang (6) showed that its application at the seedling stage caused noticeable inhibition of root growth. Initial examination of the roots confirmed the growth inhibition and also revealed a region of apparent nonenlargement of the cortex. In addition, the absence of lateral roots was apparent. Examination of the effect of alachlor on mitosis indicated possible interference with the mitotic processes.

Although probable modes of action of some chloroactamide herbicides have been reported (10), no primary mode of action has yet been determined for alachlor. The investigations carried out in this study were done to determine the anatomical and morphological effects of

alachlor on seedlings.

Materials and Methods

Oat seeds were sown in 6.5 cm diameter plastic pots filled with quartz sand. The pots were saturated by a subirrigation application of distilled water and placed in a controlled environment chamber with a 30 C-light period and a 21 C-dark period. Day length was 14 hours at a light intensity of 7000 Lux. Five days after imbibition, all pots were thinned to 4 plants approximately 3 cm in height. Treatments consisted of a daily subirrigation application of Hoagland nutrient solution (9) (half-strength) containing 10^{-4}M alachlor, 10^{-6}M alachlor, or no alachlor. Random pots were selected on a daily basis for visual and cytological examination of root growth inhibition.

Material for cytological study was fixed in ethanol, acetic acid, formalin and water (14:1:1:4, v/v/v/v) and passed through a series of N-butanol and xylene, then embedded in tissue-mat (m.p. 56 to 58°C). Material was sectioned at 5µ and stained with toluidine blue-O before removal of paraffin matrix according to McGrath (12).

Because of the difficulty of preserving cortical and epidermal tissue in paraffin, a condition not uncommon to roots (4), additional material was fixed in acrolein and embedded in glycol methacrylate. Material was sectioned

at 2μ and stained with toluidine blue-O according to the procedure of Fedder and O'Brien (8).

Treated and untreated root tips were hydrolyzed in 1N HCl for 7 minutes at 60°C. Root tips were then stained with Feulgen's Reagent and mascerated on a microscope slide for determination of mitotic indices.

Results and Discussion

The most noticeable effect of alachlor on the oat root system was that of inhibition of growth (Figure 1). Inhibition of root growth by 10^{-4}M alachlor was first apparent at about 3 days after treatment and became more pronounced with time. Inhibition of root growth also occured with 10^{-6}M alachlor, but onset was not as rapid and the degree of inhibition was not as severe. The absence of lateral roots in treated seedlings is shown in Figure 2. In addition to this, a region (5-10 mm from the root tip) of nonenlargement of cortical cells was noted. Root inhibition by alachlor, although previously reported (6, 7) was not characterized in respect to any of these differences.

Alachlor had no effect on the formation of lateral root primordia, however, the number of laterals per root was not examined. The treated primordia failed to develop further and did not elongate and rupture the cortex and epidermis (Figure 3) as normally occurs. These inhibited

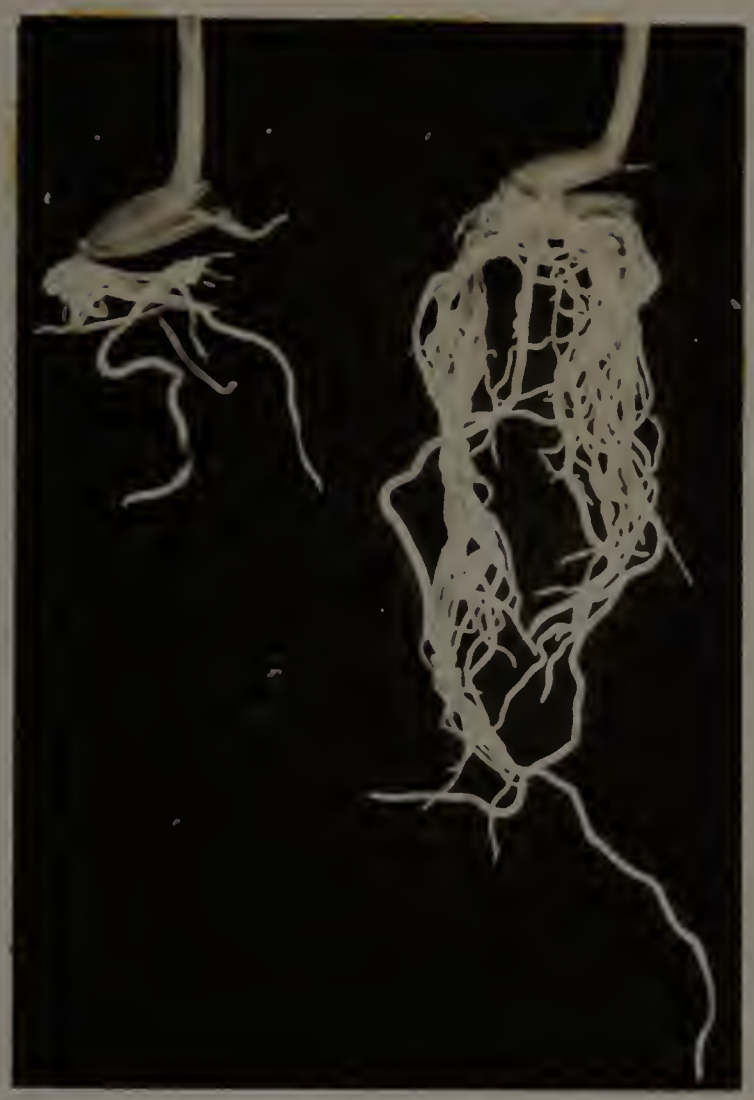

Fig. 1. seedling root system of control (right) and 10^{-4}M alachlor treated plants (left) 5 days after treatment.

Fig. 2. Alachlor (10^{-4}M) treated root system 5 days after treatment showing lack of lateral roots and zone of nonenlargement (arrow).

primordia were shown to be anatomically normal in positioning (forming opposite xylem poles), in organization and with a small root cap.

Examination of the terminal region of both treated and untreated roots showed no difference in root cap structure or, in cross section, in the differentiation of the primary root tissues.

Microscopic comparison of the region of nonenlargement

Fig. 3. Alachlor (10^{-4}M) treated root section showing formation of laterals still confined within root cortex tissue.

of cortical cells was made of the same region from treated and untreated roots (Figures 4A and 4B). Untreated roots appeared as expected, with the early loss of the epidermal layer and the conspicuous thickening of cortical cells below the original epidermis as has been reported by Avery (1). The most notable differences are in the persistance of the epidermal layer in treated roots and the enormous increase in size of cortex cells of untreated plants in comparison to treated plants. At later points in development the size of cortex cells in untreated plants is many times greater than shown in Figure 4A. Bardzik (2) has shown alachlor inhibition of cell enlargement with other tissues. He reported the lack of cell enlargement in oat

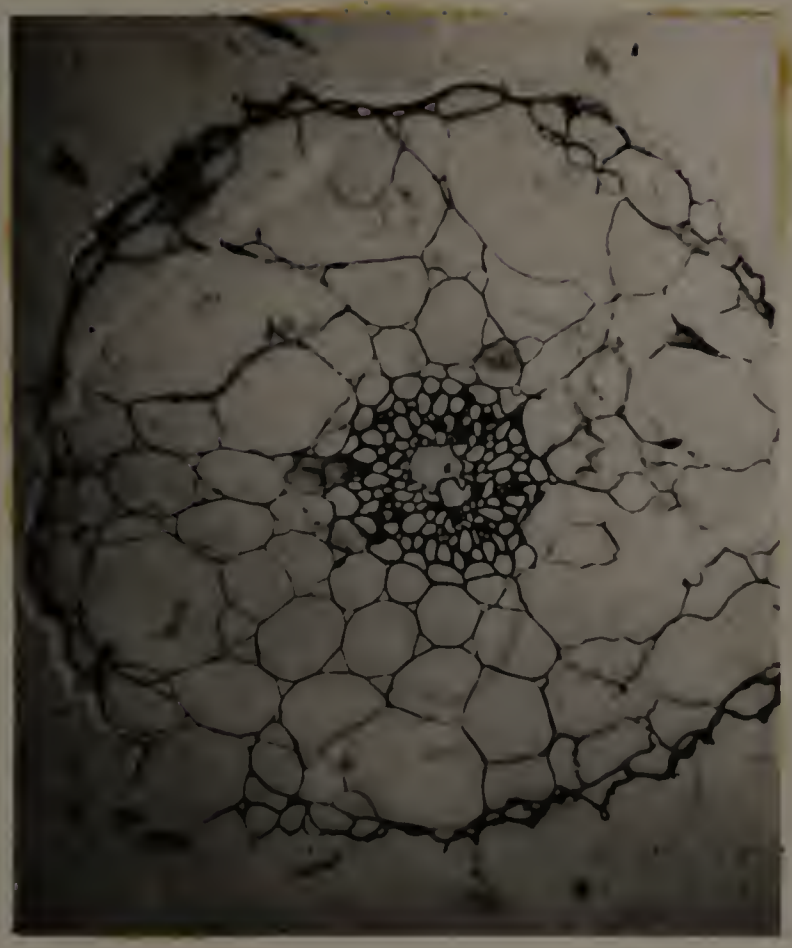

Fig. 4A. Cross section of control root showing greatly enlarged cortical cells and ruptured epidermis X100.

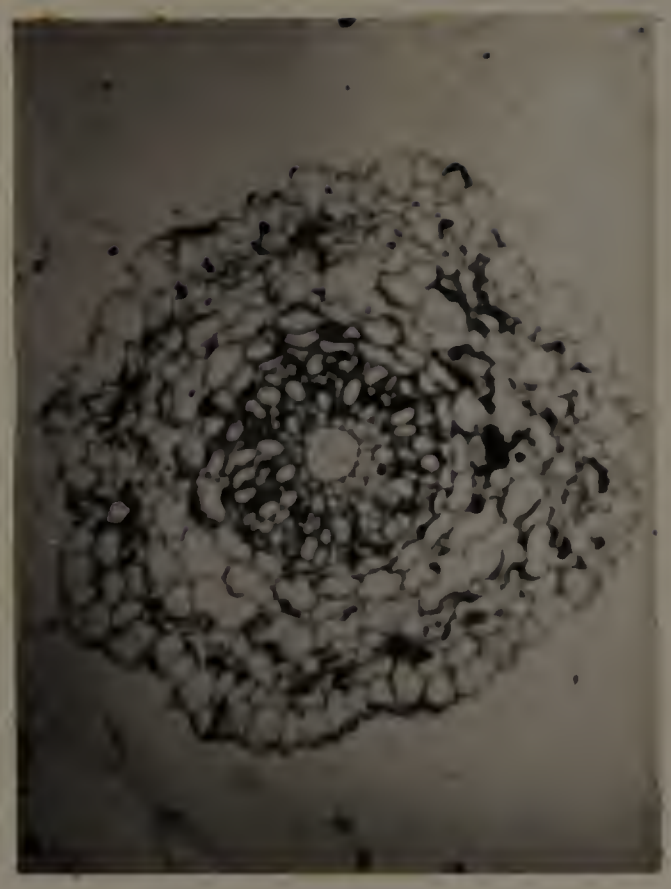

Fig. 4B. Cross section of alachlor treated (10^{-4}M) root in region of nonenlargement of cortical cells. Epidermis still intact X100.

coleoptile tissue due to alachlor.

Paraffin embedded material also displayed the same similarity of treated and untreated roots in respect to the number of cells and the degree of lignification in the xylem tissue, but comparison of cortical and epidermal tissues by this method was impossible because of poor preservation.

Roots treated for 4 days with 10^{-4}M alachlor showed a mitotic index of 7 and 2 in untreated and treated root tips, respectively. By day 7 the index for untreated root tips was 5.6, and for treated roots zero. After 7 days of treatment with alachlor, the nuclear envelopes were readily

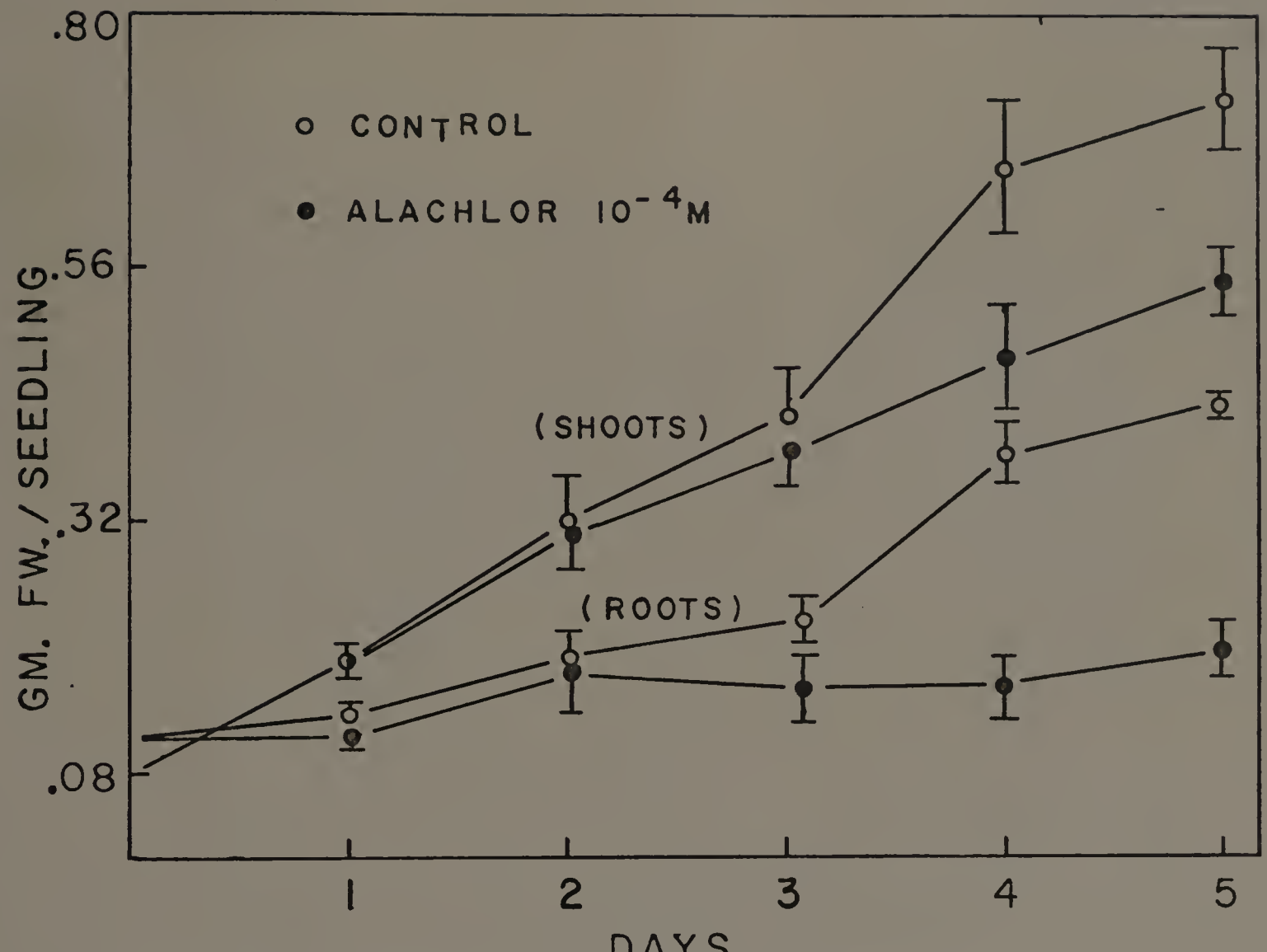

Fig. 5. Time course of increase in fresh weight for oat seedlings treated with and without alachlor (10^{-4}M).

destroyed in preparing squashes. These observations are consistent with weight measurements of intact seedlings (Figures 5 and 6). The time course of increase in fresh weight shows an alachlor-induced inhibition beginning at 4 days in shoots and at 3 days in roots. The time course of increase in dry weight shows an alachlor-induced inhibition beginning at 4 days in roots but not at all in shoots.

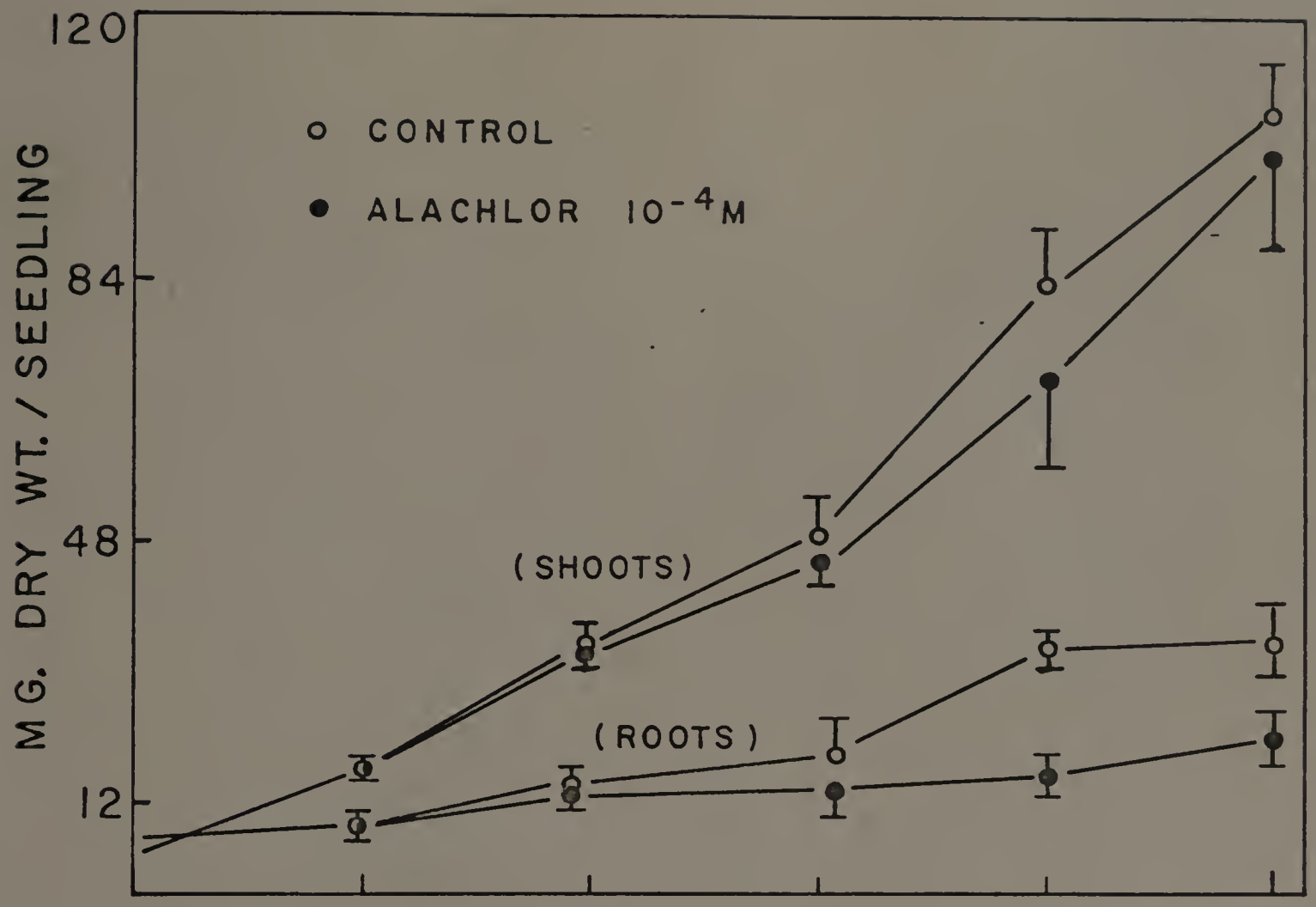

Fig. 6. Time course of increase in dry weight for oat seedlings treated with and without alachlor (10^{-4}M).

The results of these experiments suggest the site of action of alachlor is located in the root system of Avena. Chandler (5) showed that in soybean and wheat alachlor accumulated in the roots and was not translocated to the shoots. The lack of inhibition by alachlor of dry weight increase in Avena shoots indicates that the alachlor-induced inhibition of seedling height reported by Chang is not due directly to alachlor but to an inhibition of water uptake.

The occurence of inhibited water uptake in Avena seedlings by a subirrigation treatment of alachlor has been reported by Marsh et al (11). Bates (3) noted that exposing oat root sections to alachlor (10^{-4}M) resulted in strong inhibition of uptake of a broad range of solutes. This inhibition of solute uptake by the roots would result in a decrease in accompanying water uptake as well as limiting solute transport to the growing points, which would undoubtedly lead to growth inhibition.

Literature Cited

1. Avery, G., Jr. 1930. Comparative anatomy and morphology of embryos and seedlings of maize, oats, and wheat. Bot. Gaz. 89:1-39

2. Bardzik, J. M. 1974. An investigation of the metabolic and physiological effects of alachlor on Avena seedlings and coleoptile sections. Ph.D. Thesis, Univ. of Massachusetts.

3. Bates, J. R. 1975. An investigation into the mode of action of alachlor. Master's Thesis. Univ. of Massachusetts.

4. Bonnett, H. T., Jr. and J. G. Torrey. 1965. A comparison of the application of several histological procedures to mature primary root tissue. Protoplasma. 60:310-313

5. Chandler, J. M., E. Basler and P. W. Santelmann. 1974. Uptake and translocation of alachlor in soybean and wheat. Weed Sci. 22:253-258

6. Chang, T. 1973. Effect of alachlor on Avena seedlings. Ph.D. Thesis. Univ. of Massachusetts.

7. Eshel, Y. 1969. Phytotoxicity, leachability and site of uptake of 2-chloro-2',6-diethyl -N-(methoxymethyl) acetanilide. Weed Sci. 17:441-444

8. Fedder, M. and T. P. O'Brien. 1968. Plant microtechnique: some principles and new methods. Amer. J. Bot. 55:123-142

9. Hoagland, D. R. and D. I. Arnon. 1950. The water-culture method for growing plants without soil. Calif. Agr. Expt. Sta. Circ. 347

10. Jaworski, E. G. 1969. Analysis of the mode of action of alpha-chloroacetamides. J. Agr. Food Chem. 17:165-170

11. Marsh, H. V., Jr., K. Woodard and J. R. Bates. 1975. Effect of alachlor on water utilization by oat seedlings. Northeastern Weed Sci. Soc. Proc.

12. McGrath, T. C. 1971. The seedling root apical meristem of Zamia floridana. A.DC. Master's Thesis, Smith Coll.

SECTION III

INHIBITION OF SOLUTE UPTAKE BY ALACHLOR IN *AVENA* ROOT SECTIONS

Abstract

2-Chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide (alachlor) at a concentration of 0.1 mM inhibited uptake of leucine, ∝-amino isobutyric acid, 3-O-methyl glucose and potassium but not calcium. Inhibition of leucine uptake was noted within 30 minutes. Examination of leucine incorporation into protein indicated that alachlor does not inhibit this process. In addition, no effect of alachlor on respiration or on membrane leakage could be detected.

Introduction

Growth inhibition of Avena seedlings by alachlor (a chloroacetamide herbicide) has been well established by Chang (4) and Bardzik (1). The inhibition of root growth by alachlor noted by Eshel (7) has been shown to be the site of action of alachlor by Bates (2). These findings are in agreement with the findings of Chandler et al (3) who showed that alachlor once taken up by the roots of soybean or wheat remains primarily in the root system and is not translocated to the shoot in any great amount. Rao (12) working with alachlor reported that alachlor may inhibit protein synthesis. This would seem likely since other chloroacetamides such as propachlor (6) and CDAA (8) have been shown to interfere with protein synthesis. However

our preliminary experiments indicated that protein synthesis was not inhibited although leucine uptake was. It will be shown here that alachlor has no effect on leucine incorporation into protein within the time period when growth inhibition is complete by alachlor but that it does inhibit the uptake of a broad range of solutes without affecting respiration or causing membranes to become leaky.

Materials and Methods

Plant Material: Oat seedlings (Avena sativa varr. orbit) were grown on moist paper toweling or on vermiculite in plastic boxes. Seedlings were grown at room temperature in the dark. Three day old and four day old seedlings were used as a source of root and coleoptile sections respectively.

Uptake studies: Sections 1 cm in lenght (from the tip back) of three day old primary roots were weighed and placed in 25 ml beakers (15 sections/beaker) containing 1.5 ml of 10 mM potassium phosphate buffer (pH 6.5) with and without alachlor. Beakers were then placed on a shaker for various preincubation periods after which time uptake was initiated by addition of 0.1 µci of labeled solute (^{14}C-1-leucine 62 µci/mM, ^{3}H-3-O-methyl glucose 60µci/mM, and ^{14}C-alpha-amino isobutyric acid 50µci/mM). After a 15 minute uptake period, the solution was aspirated and the sections washed in 5 ml of cold distilled water for 15 minutes. The wash

solution was aspirated and the sections placed in 5 ml of scintillation fluid (Beckman Ready-Solv Solution VI) and the radioactivity determined on a Beckman scintillation counter.

Studies of ^{45}calcium (2.3mci/mg Ca^{++}) uptake were conducted by the same procedure with the exception of a 15 minute wash in 1.0 mM calcium chloride instead of distilled water.

Leucine incorporation assay: Sections 1 cm in length (from the tip back) of three day old primary roots were weighed and placed in 25 ml beakers (15 sections/beaker) containing 1.5 ml of 10mM potassium phosphate buffer (pH 6.5) with and without 0.1 mM alachlor and 0.1 µci of ^{14}C-1-leucine (62 µci/mM). Beakers were placed on a shaker (80 oscillations/min) throughout the incorporation period. At various times the incubation medium was aspirated and sections washed in cold distilled water for 15 minutes. Sections were then boiled in ethanol. The ethanol was evaporated off and the sections were then homogenized in 1.5 ml of 1N sodium hydroxide. The extent of incorporation of ^{14}C-leucine into protein was assayed according to Mans and Novelli (11). Three-tenths ml aliquots of the homogenate were spotted on 3 cm filter paper discs and air dryed. The discs were placed in an ice cold 10% TCA solution for one hour, then transferred to 5% TCA (85^{o}C) for 15 minutes, after which they were put through a series of ethanol-ether dehydration washes. Finally the filter paper discs

were air dryed and placed in 5 ml of scintillation fluid (Beckman Ready-Solv Solution VI) and their radioactivity determined. A 0.2 ml aliquot of the homogenate was placed directly into 5 ml of scintillation fluid and counted to determine total leucine uptake. The radioactivity remaining in the filter paper discs was assumed to represent leucine incorporation into protein. An additional 0.3 ml aliquot was used for protein determination by the Lowry et al method (10).

K^+ determination: Sections 1 cm in length (from the tip back) of three day old primary roots were weighed and placed in 25 ml beakers (15 sections/beaker) containing 1.5 ml of distilled water, various concentrations of potassium phosphate with and without alachlor (0.1 mM). Beakers were placed on a shaker (80 oscillations/min) for 3 hours for K^+ uptake after which the incubation solution was aspirated and the sections washed in cold distilled water for 15 minutes. The wash solution was aspirated and the sections digested using nitric acid. Tissue digests were assayed for potassium content using a flame atomic absorption spectrophotometer.

Respiration measurements: Sections 1 cm in length (cut 3 mm from the tip back) of isolated 4-day old coleoptiles were placed in warburg flasks containing 3 ml of 50 mM trizma/potassium phosphate buffer (pH 7.2) with and without 0.1 mM alachlor. Oxygen uptake was measured on a Gilson manometer.

Leakage studies: Root systems of 3-day old seedlings were excised and groups of equal weigths were placed in 25 ml beakers containing 15 ml of distilled water and 0.15M mannitol with and without 0.1 mM alachlor. Conductivity measurements were made using a Serfass conductivity bridge.

All experiments contained in this section were conducted two or more times.

Results and Discussion

Results presented in Figure 1 show the inhibitory effect of 0.1 mM alachlor on the uptake of ^{14}C-1-leucine by oat root sections. The onset of inhibition occurs between 15 and 30 minutes after exposure to alachlor. The percent inhibition increases greatly with the time of incubation up to approximately 70% inhibition at 3 hours. The increasing rate of leucine uptake in the control tissue is similar to that noted by Leonard and Hanson (9) and is the result of prolonged immersion of root sections in water.

To see if alachlor behaves like other chloroacetamides with regard to inhibition of protein synthesis, the incorporation of ^{14}C-leucine was examined. Figure 2 depicts the effect of 0.1 mM alachlor on ^{14}C-leucine incorporation in the oat root sections. As can be seen by the hypothetical curve, there is no inhibitory effect due to alachlor on ^{14}C-leucine incorporation up to 1 hour after exposure to alachlor. However, between 1 and 3 hours after exposure to

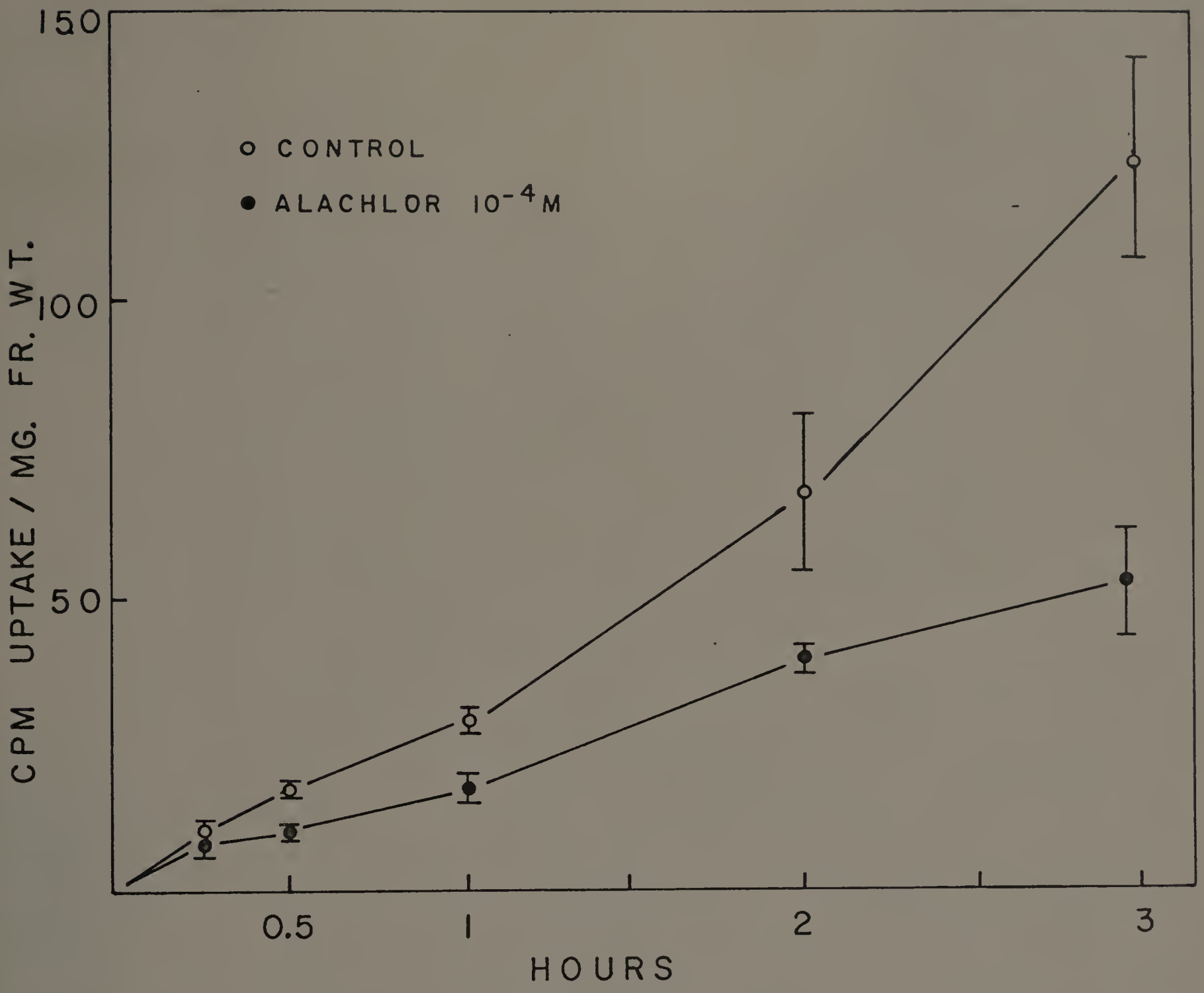

Fig. 1. Time course of leucine uptake by oat root sections with and without 0.1 mM alachlor.

alachlor there is an apparent stimulation of leucine incorporation.

In order to determine if the alachlor-induced inhibition of leucine uptake was reversible, an experiment was conducted in which root sections were exposed to 0.1 mM alachlor for one hour and then transferred to a medium free

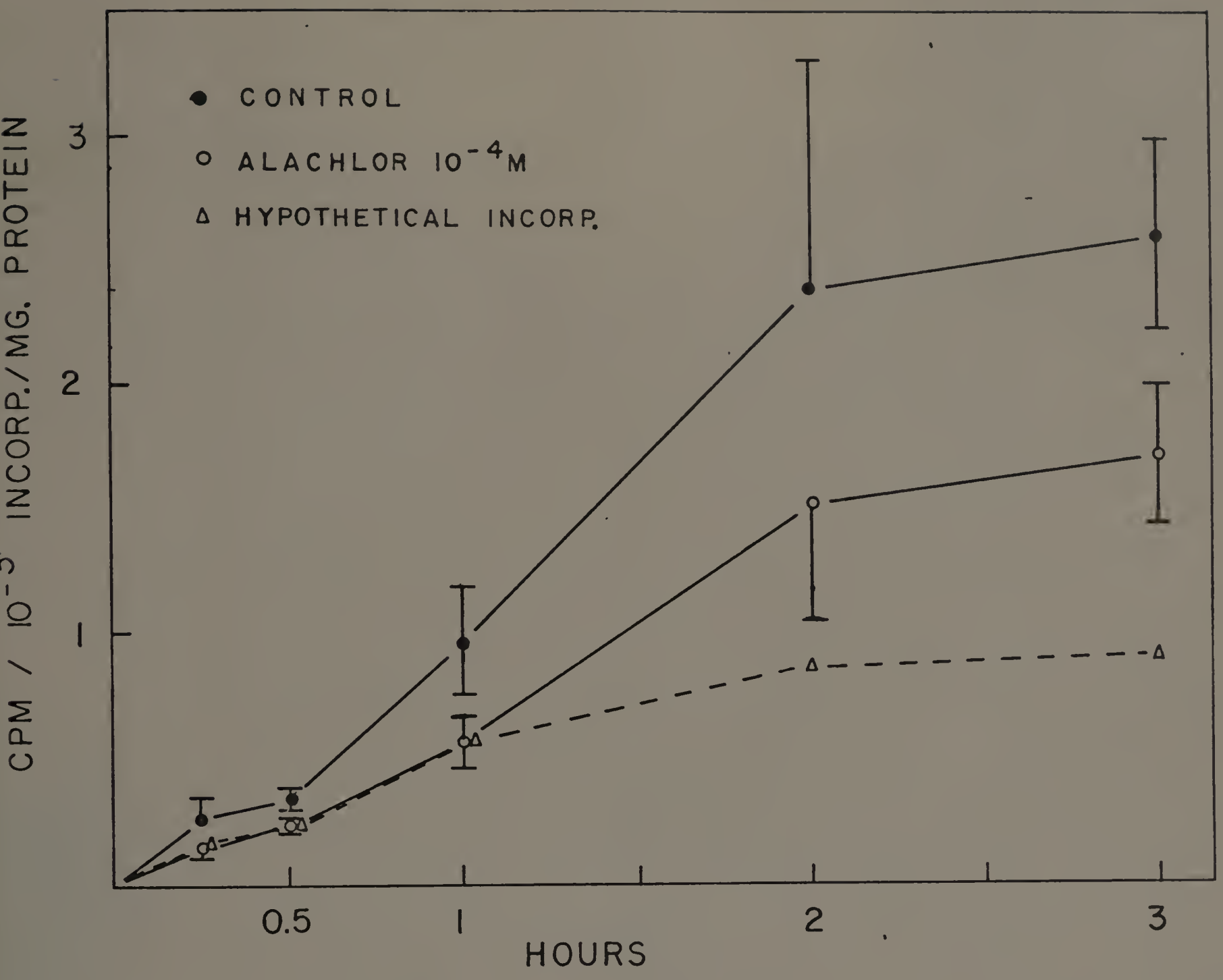

Fig. 2. Time course of ^{14}C-1-leucine incorporation in oat root sections in the presence of 0.1 mM alachlor (○) and without alachlor (●). The dotted line (Δ) represents the hypothetical amount of leucine that should be incorporated based on the amount of free leucine (cpm uptake/mg protein) in treated tissue in relation to untreated tissue.

of alachlor for a further two hour incubation. Sections were then exposed to ^{14}C-1-leucine for a 15 minute labeling period. The results of this experiment (Table 1) show

Table 1

Leucine Uptake
by Avena Root Sections

*cpm/mg. fr. wt./hr.

TREATMENTS	1 hr.	3 hr.
Control	14.7 ±1.6	30.2 ±0.4
Ala 10^{-4}M	8.3 ±0.5	____
**Ala 10^{-4}M	____	45.0 ±2.6

* Values are an average of 3 repetitions of 15 sections each.
** Exposure for 1 hr, then transferred to control conditions for remainder of 3 hr treatment.

that not only is the alachlor-induced inhibition reversible but that the washing out of alachlor has a stimulatory effect on leucine uptake.

Table 2 shows the effects of various concentrations of alachlor on the uptake of various solutes. Leucine uptake is strongly inhibited by 10^{-4}M and 5 X 10^{-5}M alachlor. In a separate experiment 10^{-6}M alachlor increased leucine uptake two-fold (data not shown). Alpha-amino isobutyric acid (α-AIB, a non-incorporated amino acid) was looked at in order to study the effects of alachlor on amino acid uptake independent of incorporation of the amino acid into protein. As seen here 10^{-4}M alachlor strongly inhibited uptake of α-AIB but 10^{-6}M alachlor has no effect. A non-metabolized sugar 3-O-methyl glucose (3-O-CH_3-Glu) was used to examine effects of alachlor on sugar uptake. Results were similar to those of α-AIB, with 10^{-4}M alachlor

Table 2

Solute Uptake
by Avena Root Sections

Treatment(3 hr)	*cpm/mg. fr. wt./hr. Leucine	-AIB	3-O-CH_3-Glu	Ca^{++}
Control	133.6 ±6.5	39.7 ±4.0	10.1 ±1.5	62.3 ±7.9
Ala 10^{-4}M	48.3 ±5.2	16.3 ±1.0	4.9 ±0.4	60.0 ±4.3
Ala $5X10^{-5}$M	79.8 ±5.6	____	____	____
Ala 10^{-5}M	225.0 ±25	____	5.1 ±0.2	____
Ala 10^{-6}M	____	32.4 ±1.0	11.1 ±3.8	61.7 ±3.4

* Values are an average of 3 repetitions of 15 sections each.

strongly inhibitory and 10^{-6}M alachlor having no effect.

Since it has been reported that alachlor inhibits chloride ion uptake in oat coleoptiles (1), the absorption of calcium and potassium were examined for possible effects by alachlor. Alachlor had no effect on calcium absorption (Table 2) at any of the concentrations tried. Results of experiments with calcium absorption under aerobic and anaerobic conditions yielded no difference (data not shown), indicating that calcium absorption in Avena roots is not an active uptake process. Additional experiments monitoring calcium wash out (data not shown) indicate that a large amount of the calcium taken up in the 15 minute labeling period may in fact be adsorbed and not absorbed. The absorption of potassium (Table 3) was inhibited by 10^{-4}M alachlor but only when the potassium phosphate concentration

Table 3

Potassium Uptake by
Avena Root Sections

*μm K^+/gr. fr. wt.

	Incubation Medium			
TREATMENT	DIST. H_2O	0.2 mM KPO_4	2.0 mM KPO_4	
			I.	II.
Original	____	____	____	44.5 ±1.5
Control (3 hr)	36.9 ±4.6	42.6 ±4.1	55.8 ±1.8	51.2 ±3.3
Ala 10^{-4}M (3 hr)	35.3 ±3.1	42.0 ±2.6	46.0 ±7.1	43.6 ±2.8

* Values are an average of 3 repetitions of 15 sections each.

in the medium was 2.0 mM. Treated and control sections both lost potassium when placed in distilled water probably due to leakage.

Examination for effects by alachlor on respiration (Figure 3) were conducted using oat coleoptile tissue. Results here show that 10^{-4}M alachlor has no effect on respiration up to 4 hours after exposure and only a slight effect at 5 hours. These results are similar to those of Bardzik (1) with alachlor and those of Duke (6) with propachlor. Additional experiments using isolated corn mitochondria showed no effect on respiration due to alachlor.

An investigation of possible alachlor-induced membrane leakage as an explaination for the inhibition of solute uptake was conducted. Root systems of 3-day old seedlings

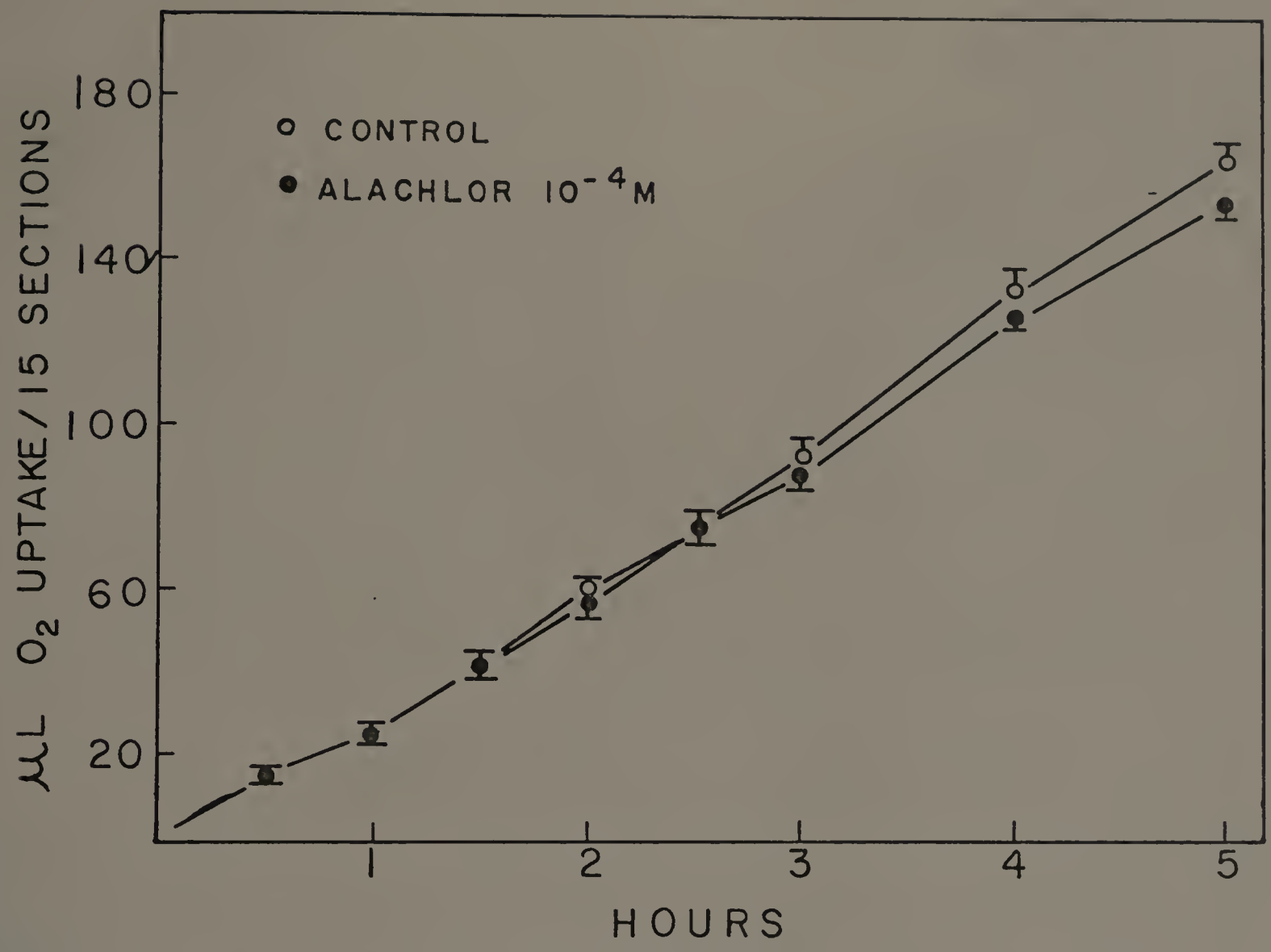

Fig. 3. Time course of O_2 uptake in Avena coleoptile sections with and without 0.1 mM alachlor.

were placed in .15M mannitol, a concentration which is far below that needed for plasmolysis (5), to prevent the leakage which results when the tissue is placed in distilled water. Ethanol and dinoseb, a known inhibitor of lipid synthesis which causes membrane leakage (13) were used as checks for membrane leakage. The results presented in Figure 4 show that even after 5 hours exposure to 0.1 mM alachlor there is no increased leakage of ions from the root sections. Additional experiments looking for an effect

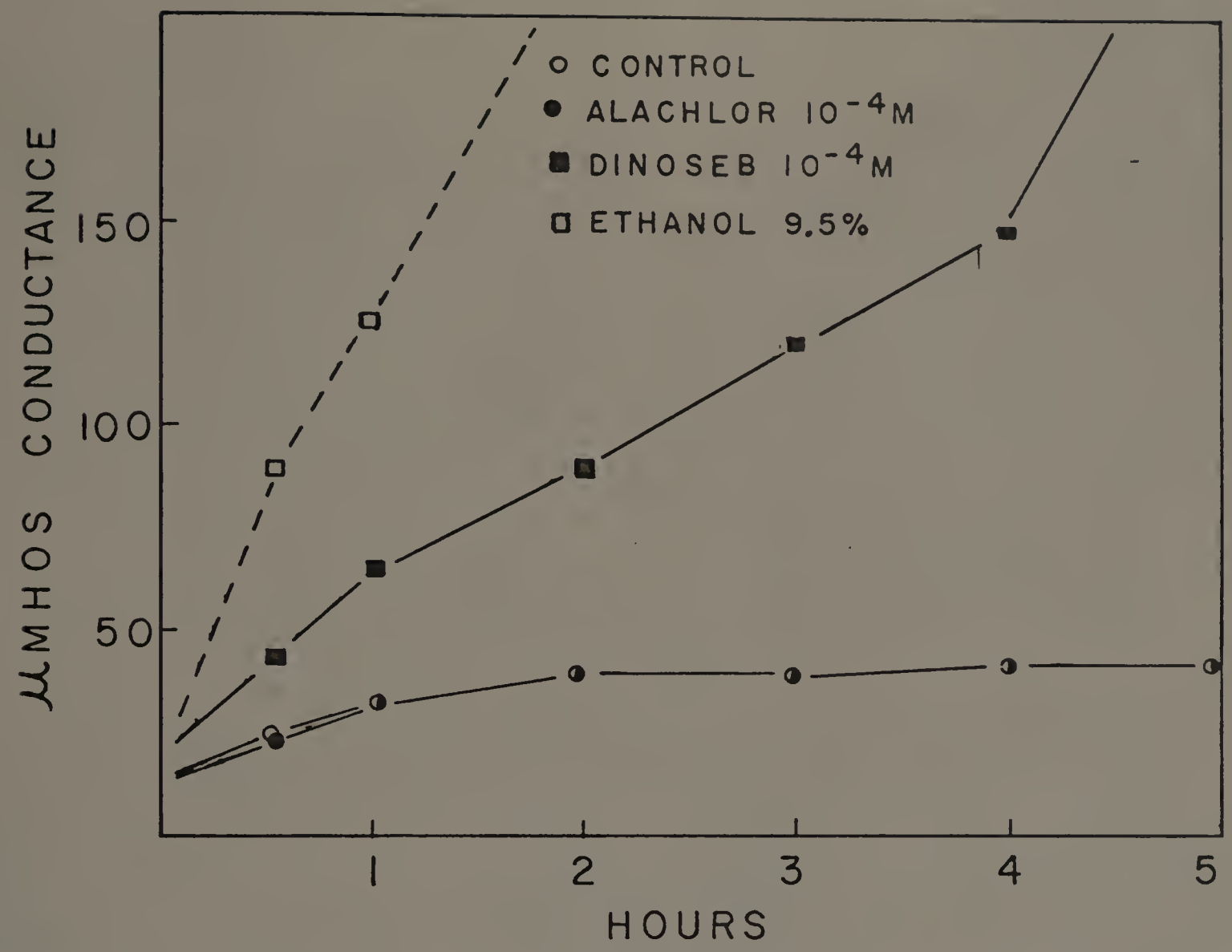

Fig. 4. Time course of electrolyte leakage from oat roots with and without 0.1 mM alachlor.

of alachlor on beta-cyanin leakage from red beets and the cyanin from sorghum seedlings yielded negative results (data not shown). Dinoseb was used as a check here also and resulted in pigment leakage.

Summary

Contrary to findings with other chloroacetamide

herbicides (8, 12), the mode of alachlor does not appear to be that of inhibiting protein synthesis. Exposure to alachlor seems to stimulate rather than inhibit protein synthesis. The stimulation of leucine uptake in root sections treated with alachlor which is subsequently washed out resembles the stimulation of uptake by low concentrations of alachlor and is probably due to low concentrations of alachlor caused by the wash out procedure. The fact that leucine uptake was stimulated by low concentrations of alachlor while the other solutes were not indicates that the stimulated leucine uptake is probably due to an apparent stimulation of leucine incorporation. The apparent stimulation of leucine incorporation may be real or may be due to an alachlor interference with leucine pools in the tissue resulting in the labeled leucine which was taken up by the treated tissue comprizing a greater percentage of leucine available for available for incorporation than in control tissue. If the apparent stimulation of leucine incorporation is not real then there is no apparent explaination for the stimulated uptake of leucine.

Inhibition by alachlor of uptake of many solutes has been shown with the exception of calcium absorption (due to calcium absorption probably being passive or adsorbed) and since no effect by alachlor on respiration or on membrane leakage could be found, it would appear that the mode of action of alachlor deals directly with solute transport systems.

Literature Cited

1. Bardzik, J. M. 1974. An investigation of the metabolic and physiological effects of alachlor on Avena seedlings and coleoptile sections. Ph.D. Thesis. Univ. of Mass. Amherst.

2. Bates, J. R. 1975. An investigation into the mode of action of alachlor. Master's Thesis. Univ. of Mass. Amherst.

3. Chandler, J. M., E. Basler, and P. W. Santelmann 1974. Uptake and translocation of alachlor in soybean and wheat. Weed Sci. 22:253-258

4. Chang, T. 1973. Effect of alachlor on Avena seedlings. Ph.D. Thesis. Univ. of Mass. Amherst.

5. Cleland, R. 1959. Osmotic concentration and auxin action. Physiol. Plant. 12:810-819

6. Duke, W. B. 1967. Ph.D. Thesis. Univ. of Illinois, Urbana, Ill.

7. Eschel, Y. 1969. Phytotoxicity, leachability and site of uptake of 2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide. Weed Sci. 17:441-444

8. Jaworski, E. G. 1969. Analysis of the mode of action of herbicidal alpha-chloroacetamides. J. Agr. Food Chem. 17:165-170

9. Leonard, R. T., and J. B. Hanson 1972. Induction and development of increased ion absorption in corn root tissue. Plant Phys. 49:430-435

10. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall 1961. Protein measurements with the folin phenol reagent. J. Biol. Chem. 193:265-275

11. Mans, and Novelli 1961. Measurement of the incorporation of radioactive amino acids into protein by a filter-paper disk method. Arch. Biochem. Biophys. 94:48-53

12. Rao, V. 1974. Mechanisms of action of acetanilide herbicides. Ph.D. Thesis. Cornell Univ. Ithaca, N.Y.

13. St. John, J. B. and J. L. Hilton 1973. Lipid metabolism as a site of herbicide action. Weed Sci. 21:477-480

SECTION IV

EFFECTS OF ALACHLOR ON CORN SEEDLINGS

Abstract

The herbicide 2-chloro-2',6'-diethyl-N-(methoxy-methyl)acet-anilide (alachlor) has been shown to severely inhibit the root and shoot growth of zero to 3-day old corn (Zea mays L.) growing in sand when applied by sub-irrigation. The roots of 5-day old seedlings were inhibited by the same treatment, but shoots of the older seedlings showed no response. The coleoptile of corn is apparently insensitive to alachlor in that the herbicide neither inhibited elongation of coleoptile segments nor ^{14}C-leucine uptake by this tissue. Leucine uptake by excised roots, however, was inhibited 40 to 67%.

Introduction

Alachlor is an effective herbicide for use in corn (Zea mays L.) where it controls the growth of most weed species with no apparent deleterious effect on the growth of the crop. Directions for the use of alachlor call for its preemergence incorporation into the top two inches of soil prior to seeding the corn, which normally is planted at a depth of 1 1/2 to 2 inches. The fact that corn is successfully grown under diverse cultural conditions employing an alachlor weed control program implies that this species is somehow resistant to the herbicide.

Experiments in our laboratory indicated that many crops are sensitive to alachlor at 10^{-4}M, which is about 40% of the concentration recommended for field application (assuming an application rate of 2 lbs. alachlor per acre). It was of interest, therefore, to establish the effects of alachlor, if any, on the growth of corn seedlings.

Materials and Methods

Corn seedling studies: Corn seeds were sown in 6.5 cm diameter plastic pots filled with quartz sand. Seedlings were grown at room temperature with a 12 hour day of 7000 Lux. Alachlor (10^{-4}M) in one-half strength Hoagland's nutrient medium was added by subirrigation to the seedlings at various times after imbibition, and exposure to alachlor was continued until termination of the experiment. Daily height measurements as well as observations of root systems were taken.

Coleoptile growth studies: Corn seeds were grown on moist paper toweling in the dark at 25°C. Four day old seedlings were harvested and 6.5 cm coleoptile sections excised. Sections were placed in 10 mM potassium phosphate buffer (pH 6.5) containing indolacetic acid (10^{-5}M) with and without alachlor (10^{-4}M). Growth was monitored with a dissecting microscope using a lens micrometer.

Leucine uptake: Dark grown seedlings (as above) were

harvested. Excised coleoptile sections (5 mm in length) and excised primary root sections (5 mm in length) were placed in beakers containing 1.5 ml of 10 mM potassium phosphate buffer (pH 6.5) with and without 10^{-4}M alachlor. One-tenth μCi of ^{14}C-1-leucine (62 μCi/mM) was added to each beaker for a period of 15 minutes, after which the sections were aspirated and washed for 15 minutes in cold distilled water. Sections were then aspirated and placed in 5 ml of scintillation fluid (Beckman Ready-Solv solution VI) and counted on a Beckman scintillation counter. Peeled coleoptiles were prepared by physically peeling away the cuticle and outer epidermal cell wall layer (3).

Results

Alachlor inhibited shoot growth of corn seedlings when applied at the time of imbibition, or 3 days after imbibition, the former treatment causing almost total inhibition and the latter approximately 40% inhibition (Fig. 1 and Figs. 2A and B). Treating the seedlings with alachlor 5 days after imbibition caused no detectable effect on the growth of the shoots. Examination of the whole seedlings revealed that the alachlor treatment at the time of imbibition or 3 days after imbibition caused a severe inhibition of root as well as shoot growth (Fig. 2A and B). In contrast to the shoots, the roots of the seedlings which had been treated 5 days after imbibition

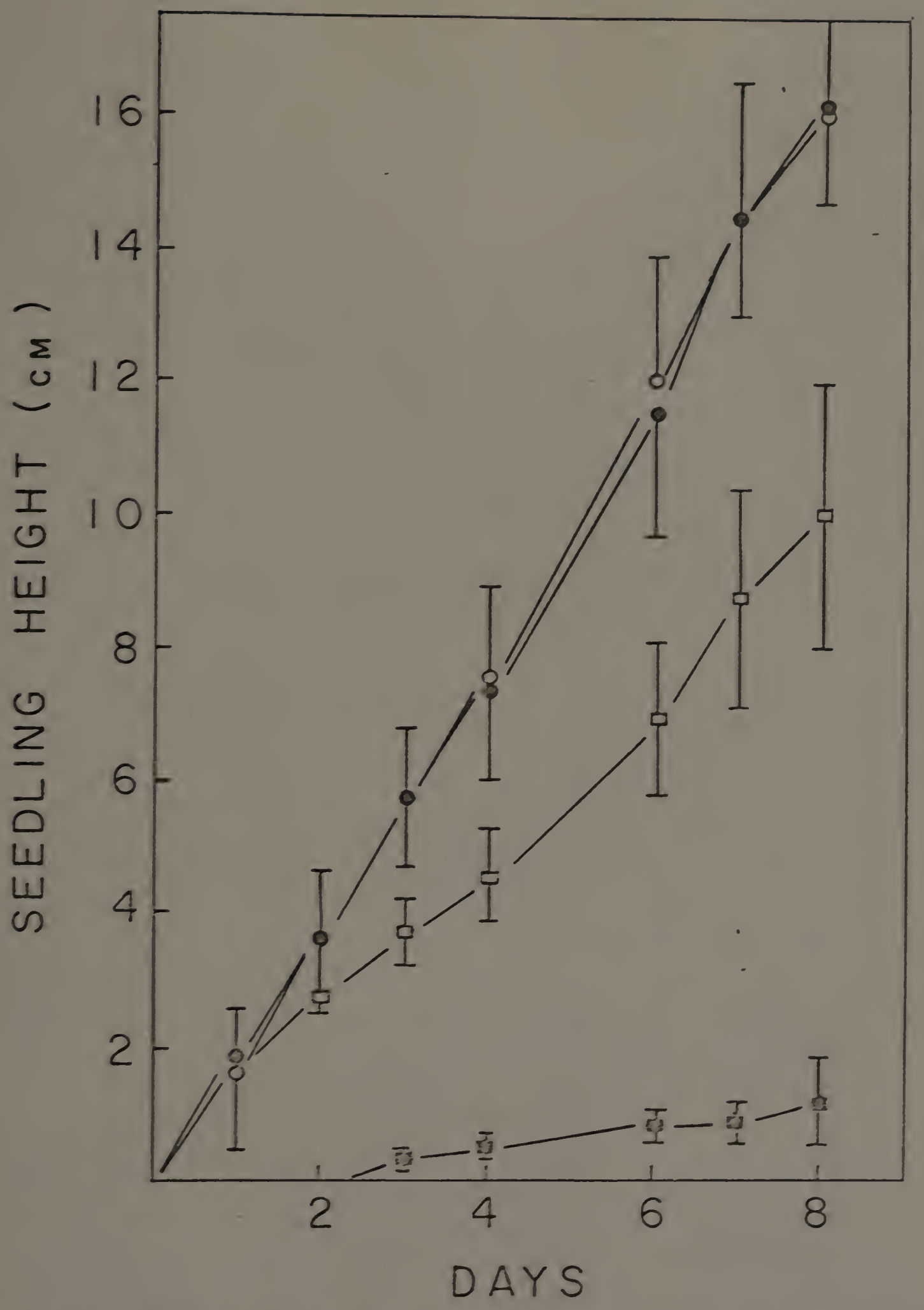

Fig. 1. Time course of corn seedling growth after emergence. Control (•), alachlor (10^{-4} M) added at imbibition (■), alachlor (10^{-4} M) added 3 days after imbibition (□) and alachlor (10^{-4} M) added 5 days after imbibition (○).

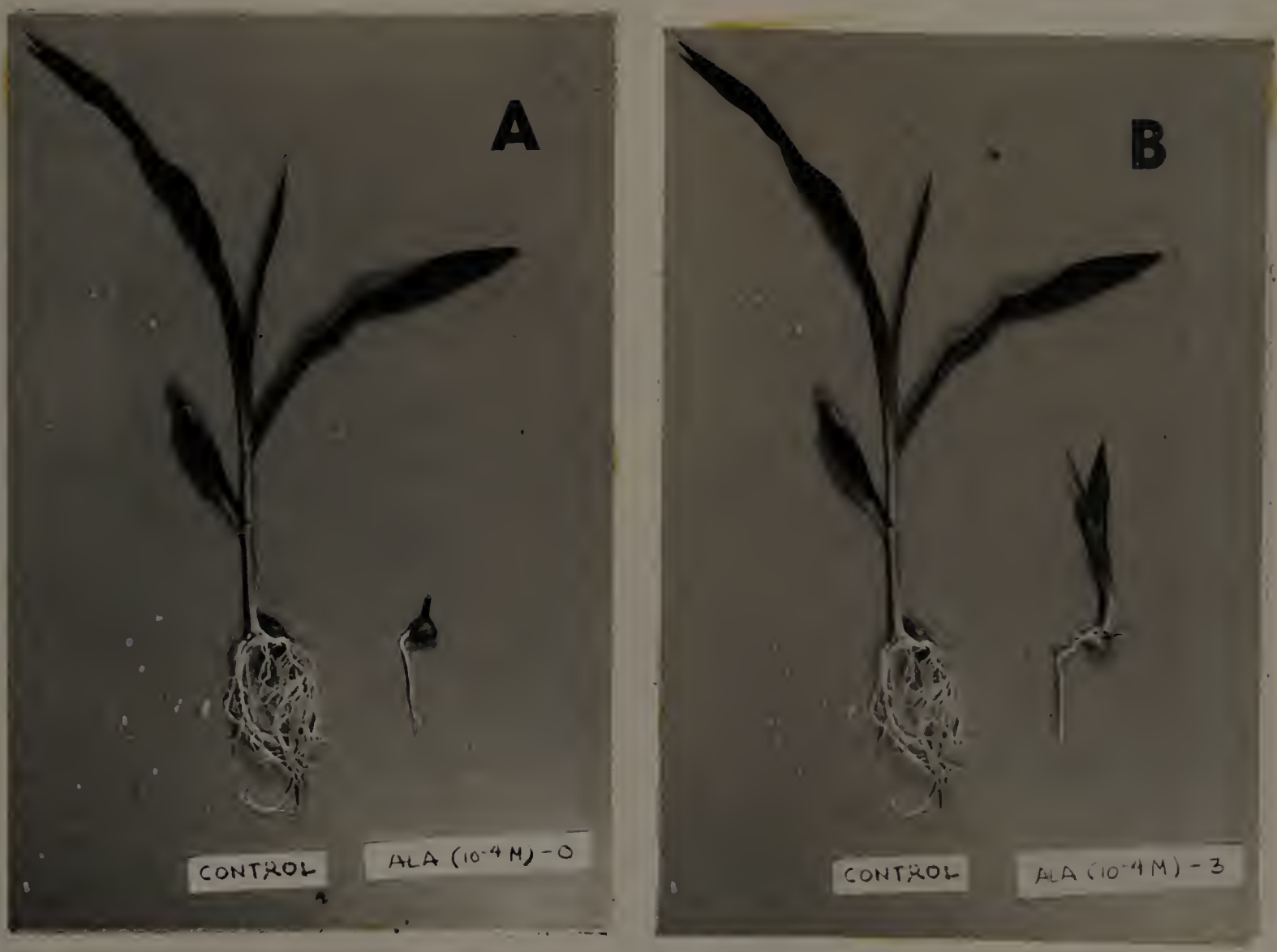

Fig. 2. Corn seedlings treated with alachlor (10^{-4}M) at time of imbibition (A) and 3 days after imbibition (B).

were also significantly inhibited (Fig. 3A), although not as severely as those treated earlier (Fig. 3B).

The effect of alachlor on the growth of corn coleoptiles was also examined since extensive work with oats has shown this organ to be especially sensitive to the herbicide (1, 2). Interestingly, elongation of the corn coleoptile appears to be completely insensitive to 10^{-4}M alachlor (Fig. 4), a concentration which causes almost complete inhibition of oat coleoptile growth after a lag period of about 2 hours (1, 2).

Fig. 3. Corn roots systems treated with alachlor (10^{-4}M) 5 days after imbibition (A) and 3 days after imbibition (B).

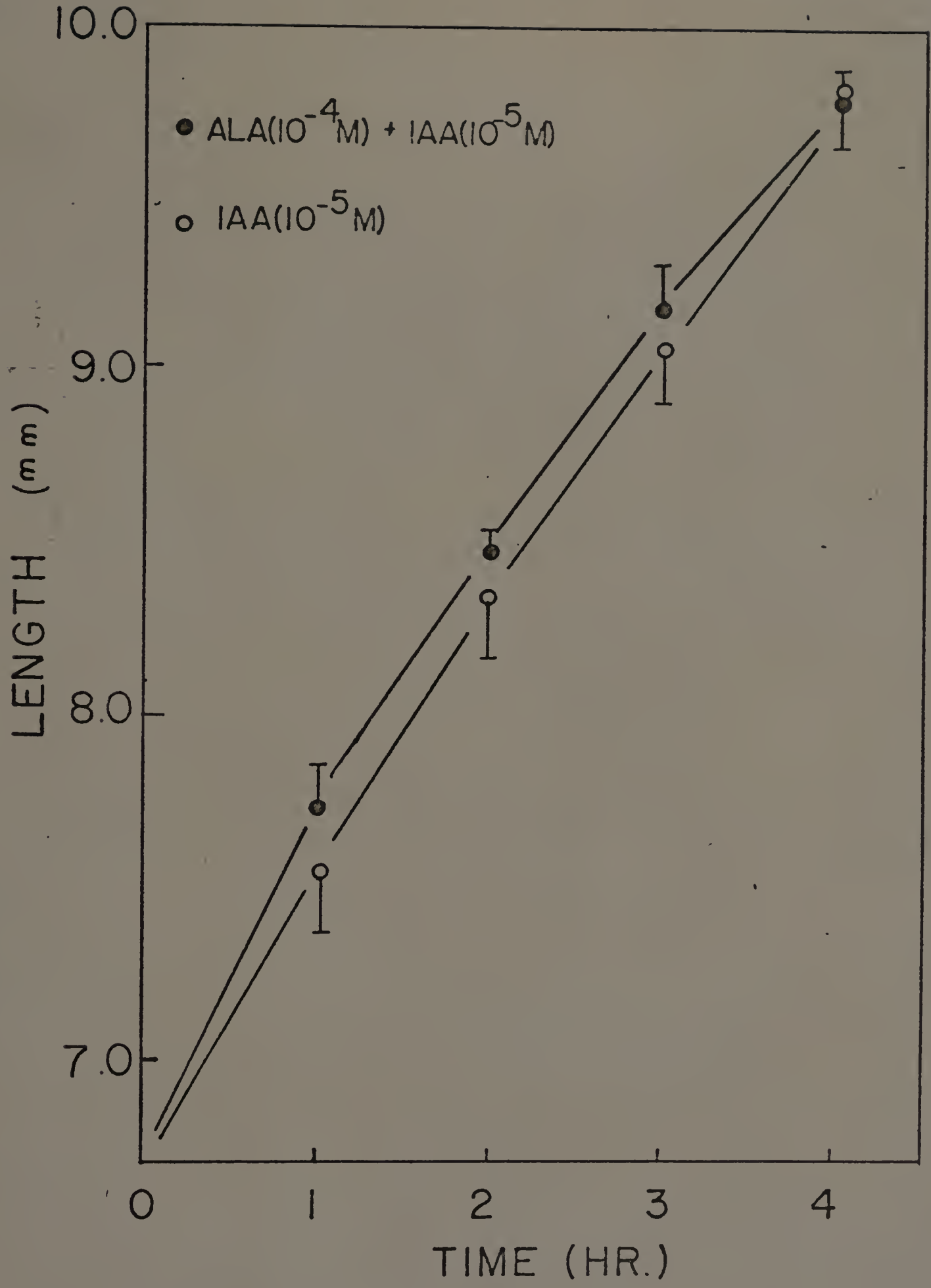

Fig. 4. Time course of auxin-induced corn coleoptile elongation in the presence and absence of 10^{-4} M alachlor.

^{14}C-Leucine Uptake by Corn Tissue

Treatment (3 hr.)	Leucine Uptake (cpm/mg. fr. wt./hr.)			
	Root Sections		Coleoptile Sections	
	I	II	Unpeeled	Peeled
Control	16.8 ±5.6	20.4 ±2.5	11.2 ±0.6	19.8 ±2.8
Alachlor (10^{-4}M)	7.3 ±0.5	12.4 ±4.5	9.6 ±0.8	18.4 ±1.6
Inhibition (%)	67	40	14	7

Alachlor at 10^{-4}M causes 40 to 60% inhibition of metabolite transport in both oat root and coleoptile tissues (2). Examination of the effect of this concentration of alachlor on ^{14}C-1-leucine uptake by root and coleoptile sections revealed that uptake by corn roots was as sensitive as in other species (Table). Leucine uptake by the coleoptiles, however, was only slightly inhibited. Since the coleoptile cuticle is a barrier to the transport of some metabolites in other tissues (3), the cuticle was removed and the effect of alachlor on ^{14}C-1-leucine uptake again monitored. The data presented in the Table indicate that although the cuticle may act as a barrier to leucine uptake, it can not be responsible for the corn coleoptile's insensitivity to alachlor.

Discussion

It has been demonstrated that under certain conditions a concentration of alachlor within the range recommended for field application can inhibit the growth of corn seedlings. That the herbicide does not normally have such drastic effects as noted here may be attributed to several factors. First, the soil probably binds some of the alachlor, thus reducing its effective concentration to the point that it is not so inhibitory to corn but still inhibits weed seedlings. Second, the corn seeds may be planted just at the lower boundary of where the herbicide is incorporated into the soil, thus the seed may escape direct contact with a significant amount of the herbicide. Thus, when the seed germinates, the sensitive roots can be envisioned as growing down out of the zone of the alachlor while the shoot, protected by the resistant coleoptile, grows up through the alachlor layer.

Literature Cited

1. Bardzik, J. M. 1974. An investigation of the metabolic and physiological effects of alachlor on Avena seedlings and coleoptile sections. Ph.D. Thesis, Univ. of Mass. Amherst.

2. Bates, J. R. 1975. An investigation into the mode of action of alachlor. Master's Thesis, Univ. of Mass. Amherst.

3. Cleland, R. 1973. Auxin-induced hydrogen ion excretion from Avena coleoptiles. Proc. Nat. Acad. Sci. 70:3092-3093.